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Full Length Research Paper

Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants

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The antimicrobial activity of the total alkaloids from five medicinal plants namely *Jatropha curcas, Calotropis procera, Magifera indica, Carica papaya* and *Psidium guajava,* commonly used in northern Nigeria for the treatment of various ailments was evaluated using disc diffusion assay. The extracts were used against three bacterial isolates and two fungal isolates including *Staphylococcus aureus, Streptococci*, *Lactobacillus spp.*, *Actinomycetes* and *Candida albicans,* respectively. The results obtained show that all the plants contains various level of alkaloids and an appreciable level of antimicrobial activities were observed at a concentration level of $6x10^2$ µg/cm^3 indicating that the plants could be a potential source of alkaloids that may be used for the treatment of various microbial diseases caused by the tested organisms.

Key words: Antimicrobial activity, total alkaloids, medicinal plants.

INTRODUCTION

Medicinal plants contain substances that can be used for therapeutic purposes or which are used as precursors for the synthesis of useful drugs (Soforowa, 1993). They are of great importance to the health of the individuals and communities and they play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents as well as important raw materials for the production of traditional and modern drugs (http;www.maphd.com/wimp.htm). The plants are rich in wide variety of secondary metabolites, which have been found *in-vitro* to have antimicrobial properties. These metabolites are low molecular weight substances which are acceptable to be essential for producing substances that are inhibitors of other organism that compete for food supply or regulators of cellular differentiation process (Demain, 1999; Demain and Fang, 2000). Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name alkaloid is derived from the word alkaline and is used to describe any nitrogen containing base (Wikipedia, free encyclopaedia). Alkaloids can also be defined as heterocyclic nitrogen compounds that are reported to be useful against Human Immunodeficiency Virus (HIV) infection (Devit et al., 1996; McMahon et al., 1995). For example, morphine and cordine alkaloids such as barbarine were found potentially to be active against trypanosomes and plasmodia (Omulokoli et al., 1997; Freiburghaus et al., 1996).

Alkaloids are also reported to have microbiocidal and anti-diarrhoeal effect due to their effect on transit time in the small intestine and their ability to intercalate with microbial deoxyribonucleic acid (DNA) (Ghoshal et al., 1996; Phillipson and Niell, 1997). The increasing use or overuse of antibiotics in the treatment of microbial infections is perpetuating bacterial resistance against available antibiotics.

Consequently, it becomes necessary to increase administered doses, combined antibiotics or provide new antibiotics with lesser tendency for pathogenic organisms to develop resistance (Agyare et al., 2006).

Approximately 20% of the world plants have been subjected to pharmacological or biological test and it could be said that natural products of plant origin are important source of constituents that could be developed into drugs, dyes, fragrance and pesticides (Hamburger and Hostattman, 1991). In this study five Nigerian medicinal plants which are often used by traditional medicine healers in northern Nigeria for one ailment or another were selected for investigation with a view of standardizing the use of these plants in folk medicine.

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Table 1. Total alkaloids extracted from plants.

Plant	Plant parts	Quantity used (g)	Total alkaloids extracted (g)
Jatropha curcas	Leaves	50	0.5
Psidium guajava	Leaves	50	0.5
Carica papaya	Leaves	50	1.0
Mangifera indica	Leaves	50	0.4
Calotropis procera	Leaves	50	0.7

EXPERIMENTAL

Plants collection

The fresh leaves of *Jatropha curcas Calotropis procera, Magnifera indica, Carica papaya* and *Psidium guajava* were collected in Afaka NDA permanent site, Kaduna, Nigeria. They were properly identified at the herbarium of the department of Biological Sciences Ahmadu Bello University, Zaria, Nigeria where voucher specimen was deposited.

Sample preparation

Each plant sample was carefully dried in the laboratory at room temperature. The leaves were then ground to fine powder with a mechanical crusher and the powder was kept in polythene bags at room temperature.

Total alkaloid extraction

The dried powder of each plant leave (50 g) was mixed with 20% alcoholic granulated and dried at room temperature before the extraction of total alkaloids. The granulated powders were extracted with benzene for 6 h. The extracts were shaken with three successive portions of 25 cm^3 of 5% sulphuric acid and decolorized by heating with activated charcoal. The hot solution was then filtered using Whatmann No.1 filter paper. The combined filtrate was acidified with ammonium solution (pH 8.5), transferred into a separatory funnel and extracted with three successive portions of 20 cm^3 chloroform and the extracts combined. The chloroform was distilled off to give the total alkaloids extract (Kokate et al., 2002). Total alkaloids extracted from the leaves were tested against the targeted microorganism using disc diffusion method. Stock solution of the extract was prepared by dissolving 20 mg of each extract in 5 cm^3 of chloroform to obtained 4000 µg/ml. Using a micropipette, 0.3 ml, 0.2 ml and 0.15 ml were separately drawn into vials and the volume adjusted to 2 ml to give approximate concentration of $6x10^2$, $4x10^2$ and $2x10^2$, respectively. A control was similarly set up using only chloroform.

Disc diffusion method

Preparation of nutrient agar 28% of the nutrient agar powder was suspended in 1 litre of distilled water. It was boiled to dissolve completely and sterilized at 121°C for 15 min to 47°C and allowed to cool and poured into sterile disposable Petri dishes.

Inoculation of organism

Clinically isolated organisms *S. aureus, Streptococci*, *Lactobacillus* spp., *Actinomycetes* and *C. albicans,* obtained from the Nigerian Army Reference Hospital Kaduna, were inoculated into nutrient broth and incubated at 37°C for 18 h. The suspension of the organism in the broth was inoculated on the nutrient agar using sterile cotton swab evenly.

Impregnation of the paper

Disc diffusion method

The paper discs were impregnated with the plant extract in concentrations of 200, 400 and 600 µg/ml and allowed to soak for 10 min. The paper discs were removed and allowed to dry and then placed on the inoculated plates with the respective microbial samples. Paper discs impregnated with chloroform were used as a negative control. The petri dishes were incubated in inverted position at 37°C for 24 h in incubator. After incubation the zones of inhibitions were measured using transparent ruler in mm (Cynthia and Callghan, 1989; Revees et al., 1999; Aneja, 2003; Yamac and Bilgill, 2006).

RESULT AND DISCUSSION

The results of the total alkaloids of *C. procera*, *M. indica*, *J. curcas*, *C. papaya* and *P. guajava* were presented in Table 1. The results show that high concentration of total alkaloids was found in *C. papaya* and *C. procera* of 1.0 nd 0.7 g, respectively. The antimicrobial activity of total alkaloids from five different plants leaves were assessed using disc diffusion method (Table 2). The results showed that total alkaloids from different plants displayed varying degree of antimicrobial activities. For instance, high activity were recorded from the total alkaloids extracted from *C. papaya*, *C. procera*, *M. indica* and *P. guajava* against *S. aureus*, *Streptococci*, *Lactobacillus* spp. and *C. albicans* with zones of inhibition ranging from 23 to 26 mm at concentration of $6x10^2$µg/cm^3 (Table 2).

Moderate activities were recorded from the total alkaloids extracts of *J. curcas*, *P. guajava*, against *S. aureus*, *Streptococci*, *Lactobacillus* spp. respectively, and no activity was recorded from the total alkaloids extracts of *J. curcas*, *P. guajava* and *C. procera* against *Actinomycetes* and *C. albicans,* respectively (Table 2). The results show that total alkaloids extracted from *C. papaya*, *C. procera*, *M. indica* and *P. guajava* were effective against most of the test microbes indicating a broad spectrum of activity.

Table 2. *In vitro* antimicrobial activities of total alkaloids extracts by disk diffusion method.

Plant	Concentration of total alkaloids x10^{2}µg/cm^3	Zones of inhibition (mm)				
		A	B	C	D	E
Jatropha curcas	6	5	15	20	N	N
	4	N	10	15	N	N
	2	N	05	10	N	N
	*C	N	N	N	N	N
Psidium guajava	6	23	18	17	23	N
	4	17	13	14	20	N
	2	12	10	10	17	N
	*C	N	N	N	N	N
Magnifera indica	6	25	25	20	10	24
	4	20	22	18	N	20
	2	15	16	14	N	15
	*C	N	N	N	N	N
Carica papaya	6	26	25	15	22	23
	4	22	21	10	17	20
	2	17	18	05	14	16
	*C	N	N	N	N	N
Calotropis procera	6	20	26	23	N	25
	4	15	22	19	N	21
	2	10	16	10	N	17
	*C	N	N	N	N	N

A = *S. aureus,* B= *Streptococci*, C =*Lactobacillus*, D= *Actinomycetes* E= *C. albicans* and *C = Control.

These alkaloids could be employed for the treatment of diseases caused by the targeted microbes. Although the activity was evaluated by disc diffusion methods, it is not always reliable as in many cases diffusion is the main barrier causing the activity.

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